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(54) Title: MATERIALS AND METHOD FOR DETECTING INTERACTION OF CFTR POLYPEPTIDES

(57) Abstract: The subject invention concerns materials and methods for detecting the interaction of CFTR proteins. In one embodiment, the method can be used to determine whether one CFTR polypeptide interacts with a second CFTR polypeptide. The subject invention also concerns materials and methods for screening for drugs or compositions that can restore or enhance interaction of CFTR proteins containing mutation(s) that reduce or prevent dimerization of the proteins. The assay of the present invention can be used to screen a large number of compounds in a high throughput format. The subject invention also pertains to host cells useful in the methods of the invention. The subject invention also concerns compositions and methods for treating patients afflicted with cystic fibrosis.

AMENDED CLAIMS

[received by the International Bureau on 31 May 2001 (31.05.01);
original claims 46-57 amended; new claims 58 and 59 added;
remaining claims unchanged (2 pages)]

42. The method according to claim 32, wherein said CFTR polypeptide comprises a mutation in the first nucleotide binding domain (NBD1).

43. The method according to claim 41, wherein said mutant CFTR polypeptide contains a ΔF508 mutation.

44. The method according to claim 32, wherein said CFTR polypeptide is a wild type CFTR polypeptide.

45. A host cell comprising a polynucleotide encoding a fusion protein comprising all or a portion of a first CFTR protein and a DNA binding domain of a transcriptional activator that can bind to a site on a detectable gene and a polynucleotide encoding a fusion protein comprising all or a portion of a second CFTR protein and a transcriptional activation domain of a transcriptional activator that can activate transcription of said detectable gene.

46. The host cell according to claim 45, wherein said host cell is a yeast cell.

47. The host cell according to claim 46, wherein said yeast cell is *Saccharomyces*.

48. The host cell according to claim 45, wherein the host cell is a mammalian cell.

49. The host cell according to claim 45, wherein said CFTR polypeptide is a mammalian CFTR polypeptide.

50. The host cell according to claim 45, wherein said CFTR polypeptide comprises amino acid residue 351 through 650 of the human CFTR protein sequence.

51. The host cell according to claim 45, wherein said detectable gene is selected from the group consisting of lacZ, *LEU2* and *HIS3*.

52. The host cell according to claim 45, wherein said DNA binding domain comprises the DNA binding domain of GAL4 protein.

53. The host cell according to claim 45, wherein said transcriptional activation domain comprises the transcriptional activation domain of GAL4 protein.

54. The host cell according to claim 45, wherein said CFTR polypeptides are mutant CFTR polypeptides.

55. The host cell according to claim 45, wherein said CFTR polypeptide comprises a mutation in the first nucleotide binding domain (NBD1).

56. The host cell according to claim 54, wherein said mutant CFTR polypeptide contains a ΔF508 mutation.

57. The host cell according to claim 45, wherein said CFTR polypeptide is a wild type CFTR polypeptide.

58. A method for treating a person afflicted with cystic fibrosis, said method comprising providing or administering to said person an effective amount of a compound that restores or enhances dimerization of CFTR polypeptide or the exit of CFTR polypeptide from endoplasmic reticulum of a cell.

59. The method according to claim 58, wherein said compound is provided by gene therapy of said person.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12N15/12 C12N15/62 C12N15/81 C12N1/19
C07K14/47 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CAB Data, STRAND, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THOREAU V ET AL: "Molecular cloning, expression analysis, and chromosomal localization of human Syntaxin 8 (STX8)" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,ACADEMIC PRESS INC. ORLANDO, FL,US, vol. 257, 1999, pages 577-583, XP002102758 ISSN: 0006-291X the whole document --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KUNZELMANN K ET AL: "Inhibition of epithelial Na+ currents by intracellular domains of the cystic fibrosis transmembrane conductance regulator." FEBS LETTERS, vol. 400, no. 3, 1997, pages 341-344, XP002163366 ISSN: 0014-5793 the whole document	
A	NEVILLE DAVID C A ET AL: "Expression and characterization of the NBD1-R domain region of CFTR: Evidence for subunit-subunit interactions." BIOCHEMISTRY, vol. 37, no. 8, 24 February 1998 (1998-02-24), pages 2401-2409, XP002163367 ISSN: 0006-2960 the whole document	
A	ANNEREAU ET AL: "Insight into cystic fibrosis by structural modeling of CFTR first nucleotide binding fold (NBF1)" COMPTES RENDUS DES SEANCES DE L'ACADEMIE DES SCIENCES. SERIE III: SCIENCES DE LA VIE,NL,ELSEVIER, AMSTERDAM, vol. 320, no. 2, 1997, pages 113-121, XP002085670 ISSN: 0764-4469 the whole document	
A	WO 94 25607 A (UNIV IOWA RES FOUND) 10 November 1994 (1994-11-10) the whole document	
P,A	HALLOWS KENNETH R ET AL: "Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase." JOURNAL OF CLINICAL INVESTIGATION, vol. 105, no. 12, June 2000 (2000-06), pages 1711-1721, XP002163368 ISSN: 0021-9738 the whole document	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ...ional Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9425607 A	10-11-1994	AU 6903594 A	21-11-1994